Laser-induced Fluorescence Imaging (LIFI) and Signature Analysis

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ABSTRACT

Fluorescence detection has been used as a field technique since the first mercury lamps were used to detect minerals. High power pulsed lasers and phototube detectors made airborne fluorescence LIDAR remote sensing practical for surveying oil spills. The USDOE Special Technologies Laboratory (STL) demonstrated its first airborne fluorosensor in 1980. The excimer laser/PMT detector system was demonstrated as an airborne method for detecting terrestrial oil spills. The advent of commercially-available, high-speed imagers allowed for terrestrial fluorescence imaging of features at high spatial and spectral resolution. STL began a program in early 1991 to build an imaging airborne fluorescence platform for environmental applications. This required the development of a ground-based capability that exists now as stand-alone technology.

Since 1991, STL has focused on Laser-induced fluorescence imaging (LIFI) and Laser-induced Fluorescence Spectroscopy (LIFS) as primary tools for trace detection remote sensing. We have focused exclusively on terrestrial and aquatic problems (not LIDARs). STL has designed, fabricated and fielded pulsed lasers and gated intensified CCD cameras to capture high resolution fluorescence imagery and spectra. Both airborne and ground-based systems were created in response to requirements in DOE Environmental Management, other DOE, and DoD programs. These signature-based efforts have included the development of several different airborne demonstration systems, and portable ground-based imaging and spectroscopy systems. Airborne platforms have used both “push-broom” and ICCD video systems. Ground-based systems have been developed for multi-spectral LIFI/LIFS collections and time-resolved imagers that determine phosphorescent lifetimes. Requirements have included surface uranium oxide survey technology, crime scene fluorescence imagers, the detection of heavy metal contamination by plant stress, and most recently, the use of genetically-modified plants and bacteria as bioreporters for contaminated soil (UXO’s and heavy metals).

While fluorescence remote sensing has been repeatedly demonstrated, the acceptance of fluorescence remote sensing has always been measured against passive reflectance technologies as a remote standard, and point sampling as a definitive characterization method. The successful fluorescence niche may be best described as the place where the active sensor has been designed to provide a degree of characterization that justifies the use of an active light source. These justifications range from the ability to perform wide area searches, to the need to keep personnel out of a particular area such as an active mine field.

Figure 1 shows a simplistic diagram of the graded approach to footprint reduction. At extremely close distance (point sampling), the pixel is very small at ground level so many samples are required to spatially characterize a large area. As the sensor becomes more remote, the effective pixel size or the number of pixels increases as a function of distance. At long stand-off distances, passive technologies usually dominate and provide a cost effective, wide area search for anomalies. Unambiguous characterization is difficult and false positives may limit the wide area search to presumptive classifications. However, identified regions of interest can be used to locate point sampling or laser-based sensors.

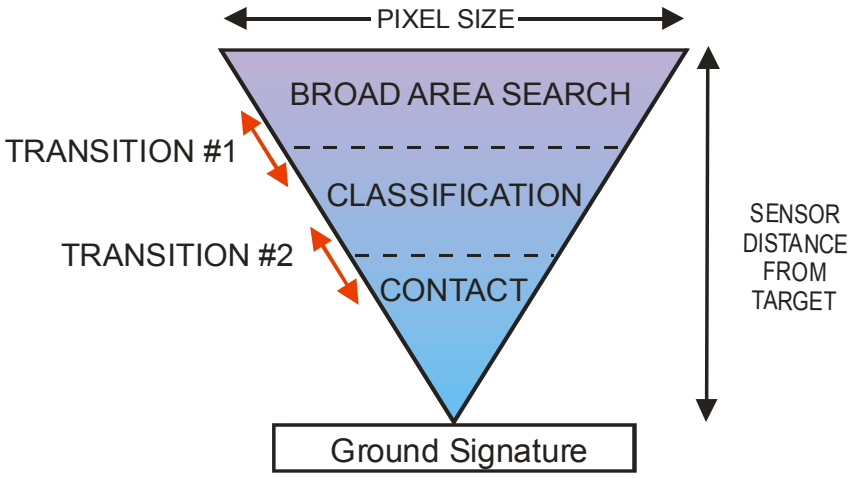

Figure 1: Graphical representation of sensor footprint and standoff.

Imaging fluorescence remote sensing finds its place in the intermediate distances and characterization levels. Both imaging and high spectral dispersion across a spectrograph limit sensitivity compared to a phototube detector, but the trade-off is justified by the increase in information. Canopy-level and airborne fluorescence can complete the footprint reduction in scenarios where passive technologies can not provide complete characterization of trace species.

Figure 1: STL LIFI system.

Systems should always be designed for maximum flexibility in the types of materials detected, however the signature types, signal strengths, size, cost and user profiles can constrain the designs for specific applications. Figure 2 shows the STL LIFI system used for both multi-band fluorescence imagery and time-resolved imagery. When collecting multi-band fluorescence, a filter wheel is used. The laser and camera components are modified commercial-off-the-shelf (COTS), but the data acquisition and processing systems were custom designs for these applications. The system is designed to be very user friendly in the field, with a limited interface for an already-taxed technician/scientist. Components are literally bolted together for rapid servicing by vendors. The data is recorded on a PC card that is used in a laptop computer for post-processing in the field.

Multi-band LIFI data is collected as a series of spectral or temporal bands that are processed using techniques similar to multi-spectral analysis. In the ground-based systems, laser-induced fluorescence spectroscopy (LIFS) is often collected simultaneously with imagery to record the fluorescence spectra (400-750 nm) of a single feature in the outdoor scene. This has proven to be a powerful, complimentary diagnostic for vegetation when combined with more traditional reflectance measurements and laboratory fluorescence spectra. With a laser excitation pulse of approximately 7 nanoseconds, the vegetation fluorescence is prompt so there is no advantage to time-resolved measurements *at the canopy level*. Electronic shuttering or "gating" of the camera's micro channel plate reduces the camera exposure to less than 50 nanoseconds. Gating can be used for isolating objects from more distant backgrounds, but for the most part, imagery is collected with short gates to reject as much ambient light as possible. While reflectance values typically vary from a few percent to 60-70 percent, fluorescence yields can vary over many orders of magnitude. This is one reason why fluorescence system development is often focused on a single need (or phenomenon) such as measuring vegetation stress. System optimization often requires trading the degree of spectral, temporal and spatial resolution against the required signal strength for good statistics.

In response to requirements of the DOE Environmental Management Program (EM-50), STL developed two related projects in fluorescence detection. One project focused on the development of systems that could detect uranium oxide surface contamination. This instrument development program was managed so that it provided technology appropriate for the vegetation project as well. The second project, "Remote Sensing of Plant Signatures", was an effort to carefully measure the response of vegetation to heavy metals in the soil. The project was also known as the "EPCOT" project because fieldwork and research was conducted at the Land Pavilion in Florida with EPCOT scientists. The Land Pavilion provided state-of-the-art greenhouse facilities and necessary resources for researchers to conduct data "campaigns" on large numbers of plants grown under controlled conditions. The hydroponic greenhouses provided the ideal setting for controlling metal contamination at trace levels (1 PPM to 100 PPM). There would often

be several universities participating with DOE and NASA labs, allowing for multiple reflectance and fluorescence acquisitions on the plants.

The work at EPCOT showed that the metal stresses investigated produced reproducible changes in reflectance and fluorescence. The changes in both spectra were generic. The emission peaks in the plant spectrum would vary in relative intensity, but no new peaks or significant shifts in peak position would occur. We felt that a more specific indicator would be required if regulators would use remote sensing data to make decisions about the presence of contamination in soil.

Figure 2: Tobacco plants inoculated with GFP-TMV

In 1998, STL made its first measurements on genetically modified plants. Figure 3 shows two photographs of a pair of tobacco plants. The plant on the right of each image has been infected with a GFP-modified virus, while the plant on the left shows the control plant. The left image shows the pair of plants under white light while the image on the left shows the plants illuminated with blue light, viewed through a green filter. The viral spreading through the leaf can be easily observed as the virus is in high concentration in the leaves.

Soon after this work, we began collaborations with Oak Ridge National Laboratories on genetically modified bacteria. ORNL had developed a bacterium that expressed GFP when exposed to extremely low levels of aromatic hydrocarbons. This work, by Dr. Robert Burlage, formed the basis for the ORNL Microbial Mine Detection System, or MMDS. STL provided fluorescence imaging support for a series of field tests that showed that mines could be detected and located by spraying a field with the benign soil bacteria, waiting several hours, and illuminating the ground with a LIFI system. Trace concentrations of TNT on the surface caused to bacteria to produce GFP. STL and other collaborators have continued to investigate the concept of "bioreporting" organisms. Recent efforts have been on GFP-modified plants with Dr. Neal Stewart presently at the University of Tennessee. We are investigating the emission properties of several plants in an effort to model the brightness and contrast required to successfully produce a system that can detect trace contaminants such as TNT, heavy metals and relevant chemical and biological materials.

Genetically modified (GM) organisms offer an extraordinary opportunity for remote sensing of the environment. Traditionally, we have followed a model where signatures in the environment are exploited by electro-optical (E-O) sensor system. Advances in sensors produce advances in signature fidelity, and dramatic increases in characterization performance. Hyperspectral passive remote sensing is a good example of how E-O technology improvements can leads dramatic increases in signature discovery and exploitation. In contrast, bioreporters allow for a degree of *signature creation*. The inducible biological signature and the sensor can finally be optimized for each other. This combined *biophotonic* sensor, both biological and electro-optical, can be tuned for maximum contrast in a particular environment.

STL is developing a model that includes the E-O system, the biological system, and the environment or scenario. The model allows for back calculating the required performance of the optical and biological performance, giving the microbiologist and the E-O designer specifications to work towards *concurrently*. The model is being built with inputs from field data as well as laboratory spectra.

Figure 4 shows a nadir view of a GM-modified Canola plant illuminated with 390 nm light. The image is a false-color representation of three bands of fluorescence imagery (R = 680nm, G = 525 nm, and B = 480 nm). This plant is one of a series that showed partial expression in the younger leaves even though the plants are part of a series that should express equally without the addition of any contaminant. The "always-on", partially expressing plants were imaged with a set of plants that had not been modified. Note that the youngest leaf shows 525 nm brightness than the other leaves of the partially transformed plant because it has more GFP fluorescence intensity respectively.

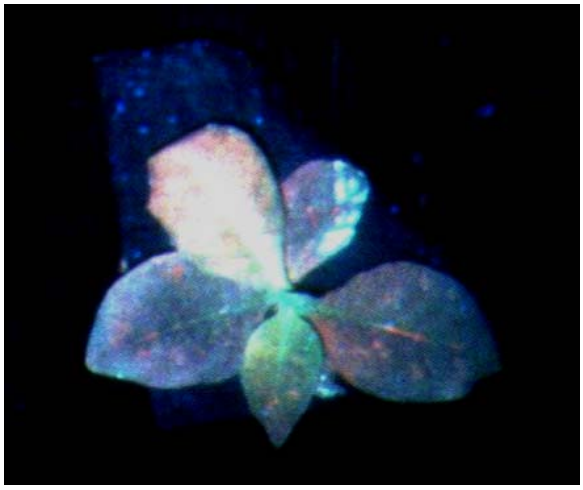

Figure 2: RGB image of GM Canola plants

If the leaves were all expressing equally, a more simple input of the leaf area index or LAI could define how many photons were reaching the bioreporter in canopy. However, in this case, the leaves are partially expressing per leaf area, and not all leaves are expressing. The model must account for the percent expression per leaf area and fractional number of leaves that express the GFP. The model will be used to specify the requirements for members for the biological team in terms of required expression.

Without modeling and field testing, the biological side of the biophotonic team does not know the true remote sensing specification required for detection. Electro-optic designers will receive continual feedback on the expression performance as that the OE system created will be effective.

This is the approach STL has chosen for designing effective fluorescence remote sensing platforms. We are using a concurrent engineering approach that is heavily influence by iterative field tests and a robust model that incorporates field experience during the development process. The model will be used in system design and as a predictive tool for designing field collections for specific trace measurements. Eventually the radiometric model will be configured as a plug-in into image processing software used by our customers and collaborators.

We will continue to gather as much signature experience as possible through ground-based measurements at close distance. Field work allows for the testing of processing algorithms that will be used to isolate the GFP signature and these tests will be used to uncover spectral interferants early in the process so that the emission and excitation wavelengths used can be appropriately adjusted. Ground-based measurements will continue to play a role as a method to test airborne concepts in a cost effective manner, and act as a stand alone technology that will be used when remote ground-based sensing is considered a better alternative than flight systems.